



## Pre-made Lentiviral Particles for GFP\_lacZ fusion

Cat#	Product Name	Amounts
<a href="#">LVP021</a>	GFP-lacZ (his) particles	200ul, $\sim 1 \times 10^7$ IFU/mL in DMEM containing 10% FBS and 60ug/ml polybrene
<a href="#">LVP021-PBS</a>	GFP-lacZ (his) particles, in vivo ready	200ul, $\sim 5 \times 10^7$ IFU/mL in PBS solution

**Storage:**  $< -70^\circ\text{C}$ , avoid repeat freeze/thaw cycles. Stable for 6 months at  $< -70^\circ\text{C}$

### Product Description:

GenTarget's lentivector system is Human Immunodeficiency Virus-1 (HIV) based plasmids for gene expression and knockdown. The lentivectors are used to generate lentiviral particles (lentivirus) that can be transduced into almost all kinds of mammalian cells, including stem cells, primary cells, and non-dividing cells both *in vivo* and *in vitro*. Lentiviral Particles stably integrate into the transduced cells' genome for long term expression, making it a great gene transfer agent.

Pre-made **GFP\_lacZ fusion** Lentiviral particles are generated from GenTarget's [SureTiter lentiviral system](#), expressing **GFP** and the **full-length Beta galactosidase (lacZ)** fusion gene under suCMV promoter. Both GFP and lacZ are fully functional. GFP-LacZ fusion was expressed with C-term His-tag. A **RFP** was bibicistronically expressed under the same suCMV promoter for titer monitoring. **So they are triple signal particles**, with GFP, RFP signals that can be visualized via microscope, and with lacZ signal via staining. A blasticidin gene under PGK promoter allows to select stable cells for long term expression.

VSV-G pseudotyped lentiviral particles are generated in 293T cell. The particles are provided in two formats:

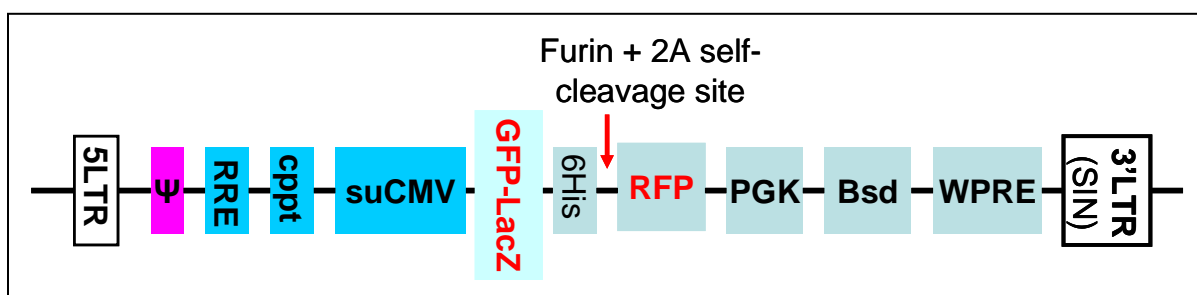
- packaged in 10% of FBS in DMEM containing 10% FBS and 60ug/ml of polybrene (Cat#: LVP021);
- packaged in PBS solution (Cat#: LVP021-PBS);

The particles in PBS solution are best suitable for serum-free cultures or for hard-to-transduced cell types. For more details about premade particles, please see [FAQ for pre-made lentiviral particles](#) (.pdf).



## Key features:

1. Lentiviral particles contain RFP-blasticidin resistant gene, allowing to generate GFP-lacZ fusion stable cell lines by Blasticidin antibiotic selection or via fluorescent cell sorting. It is actually triple signals (GFP, LacZ and RFP) particles.
2. GFP fused with full length LacZ ( $\beta$ -Galactosidase) was expressed with a C-term His-tag.
3. The strongest suCMV promoter make the pre-made virus a ideal tool for mammalian protein expression, stable cell line construction and enzymatic assays both in vivo or in vitro (see schematic vector map below).
4. **The lentivirus are ready and easy to use, simply add 50ul into your cell culture.**



## Transduction Protocols:

### 1) Transduction Protocol for Adhesive cells :

**Note:** Pre-made lentivirus is provided ready to use, so it can be simply added into your cell culture; the amount of virus to add depends on cell type. For quick transduction, add 50  $\mu$ l of virus into each well of 24-well-plate where cell density is 50% to 75%. After 72 hours (no need to change medium), visualize positive transduction rate by fluorescence microscopy. For stable cell line generation, pass cells into medium containing antibiotic or perform fluorescence cell sorting followed by antibiotic selection.

#### **Day 0:**

Seed cells in complete medium at the appropriate density and incubate overnight.

**Note:** at the time of transduction, cells should be 50%-75% confluent. For example, seed HeLa cells at  $0.5 \times 10^5$ /ml x 0.5ml in a well of a 24-well plate.

#### **Day 1:**



- Remove the culture medium and add 0.5ml fresh, warm, complete medium.
- Thaw the pre-made lentiviral stock at room temperature and add the appropriate amount of virus stock to obtain the desired MOI.
- Return cells to 37°C, CO<sub>2</sub> incubator.

**Note:** Try to avoid freezing and thawing. If you do not use all of the virus at one time, you may re-freeze the virus at -80 °C for future use; virus titer will decrease by ~10% for each freeze/thaw cycle.

### Day 3:

At ~72hr after transduction, check the transduction rate by fluorescence microscopy or calculate the exact transduction rate by flow cytometry (FACS or Guava).

### Day 3 + (optional):

Sort transduced cells by FACS, and select for antibiotic resistance. A pilot experiment should be done to determine the antibiotic's kill curve for your specific cell line (refer to the pertinent literature on generation of stable cell lines).

## **2) Transduction Protocol for Suspension Cells:**

Grow cells in complete suspension culture medium; use a shaking flask in a CO<sub>2</sub> incubator if necessary.

Measure cell density. When density has reached  $\sim 3 \times 10^6$  cells/ml, measured viability should be > 90%. Dilute cells into  $1 \times 10^6$  cell/ml in complete medium.

### Day 1:

- Thaw lentiviral particles at room temperature.
- Add premade lentiviral particles into the diluted cells at a ratio of: 50 to 100  $\mu$ l virus per 0.5 ml of cells (Note: depending on cell type, you may need to use more or less virus).
- Grow cells in a shaking flask in a CO<sub>2</sub> incubator.

### Day 2:

At 24 hours after transduction, add an equal amount of fresh medium containing relevant antibiotics. **Note:** amount of antibiotic depends on cell type. Continue growing cells in CO<sub>2</sub> incubator.

### Day 3:



At 72 hours after transduction, check fluorescence with a fluorescence microscope or calculate the transduction efficiency using a cell sorter such as FACS or Guava. Sort for fluorescence positive cells and maintain antibiotic selection to generate a stable cell line.

**Note: Filter wavelength settings:**

**GFP** filter: ~Ex450-490 ~Em525;

**RFP** filter: ~Ex545 ~Em620;

## Safety Precaution:

GenTarget lentiviral particles adapt must advanced lentiviral safety features (using the third generation vectors with self-inactivation SIN-3UTR), and the premade lentivirus is replication incompetent. However, please use extra caution when using lentiviral particles. Use the lentiviral particles in Bio-safety II cabinet. Wear glove all the time at handling Lentiviral particles! Please refer CDC and NIH's guidelines for more details regarding to safety issues.

## References:

1. [NIH stem cell training program \(Link\)](#).
2. NIH Guidelines for [Biosafety Considerations for Research with Lentiviral Vectors](#). (Link).
3. [CDC guidelines for Lab Biosafety levels \(Link\)](#).

## Warranty:

**This product is for research use only.** It is warranted to meet its quality as described when used in accordance with its instructions. GenTarget disclaims any implied warranty of this product for particular application. In no event shall GenTarget be liable for any incidental or consequential damages in connection with the products. GenTarget's sole remedy for breach of this warranty should be, at GenTarget's option, to replace the products.

**Related Products:** GenTarget's pre-made lentivirus product category.

<b>Product Category</b>	<b>Product Description (please click category name to see product's pages)</b>
<a href="#">Human, mouse or rat ORFs</a>	Premade lentivirus expressing a <b>human, mouse or rat</b> gene with RFP-Blasticidin fusion dual markers.
<a href="#">Fluorescent markers</a>	Premade lentivirus expressing human codon optimized fluorescent protein, <b>GFP / RFP / CFP / BFP / YFP</b> .



<a href="#">Luciferase expression</a>	Premade lentivirus for all kinds of luciferase protein expression: <b>firefly and Renilla</b> with different antibiotic selection markers.
<a href="#">CRE recombinase</a>	Premade lentivirus for expressing <b>nuclear permeant CRE</b> recombinase with different fluorescent and antibiotic markers.
<a href="#">LoxP ColorSwitch</a>	Premade lentivirus expressing "LoxP-GFP-Stop-LoxP-RFP" cassette, used to monitor the CRE recombination event in vivo.
<a href="#">CRISPR /hu CAS9</a>	Preamde lentivirus express humanized wild-type <b>Cas9</b> endonuclease for genomic editing with <b>CRISPR</b>
<a href="#">TetR inducible expression repressor</a>	Premade lentivirus expressin <b>TetR</b> (tetracycline regulator) protein, the repressor protein for the inducible expression system.
<a href="#">iPS factors</a>	Premde lentivirus for human and mouse iPS ( <b>Myc, NANOG, OCT4, SOX2, FLF4</b> ) factors with different fluorescent and antibitoic markers
<a href="#">T-antigen Expression</a>	Express <b>SV40 large T antigen</b> with different selection markers
<a href="#">Cell Organelle imaging</a>	Premade lentivirus for cell organelle imaging. The fluorescent marker <b>GFP/RFP/CFP was sub-cellular localized</b> in different cell organelle for living cell imaging.
<a href="#">LacZ expression</a>	Express different full length <b><math>\beta</math>-galactosidase (lacZ)</b> with different selection markers
<a href="#">Anti-miNA lentivirus</a>	Pre-made lentivirus expression a specific <b>anti-miRNA</b> cassette.
<a href="#">Fluorescent-ORF fusion</a>	Pre-made lentivirus expression a " <b>GFP/RFP/CFP-ORF</b> " fusion target.
<a href="#">Pre-made shRNA lentivirus</a>	Premade shRNA lentivirus for knockdown a specific genes ( <b>P53, LacZ, Luciferase</b> and more).
<a href="#">microRNA and anti-microRNA lentivirus</a>	Premade lentivirus expression human or mouse <b>precursor miRNA</b> . And <b>anti-miRNA</b> lentivector and virus for human and mouse miRNA.
<a href="#">Negative control lentiviruses</a>	Premade <b>negative control lentivirus with different markers</b> : serves as the negative control of lentivurs treatment, for validation of the specificity of any lentivirus target expression effects.
<a href="#">Other Enzyme expression</a>	Ready-to-use lentivirus, expressing <b>specific enzymes</b> with different selection markers.